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1 **Surface disinfection challenges for *Candida auris*: an *in vitro* study**

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1 **Abstract**

2 The emerging pathogenic multidrug-resistant yeast *Candida auris* is an
3 important source of healthcare–associated infections and of growing global
4 clinical concern. The ability of this organism to survive on surfaces and
5 withstand environmental stressors creates a challenge for eradicating it from
6 hospitals. A panel of *C. auris* clinical isolates was evaluated on different
7 surface environments against the standard disinfectant sodium hypochlorite
8 and high level disinfectant peracetic acid. *C. auris* was shown to selectively
9 tolerate clinically relevant concentrations of sodium hypochlorite and peracetic
10 acid in a surface dependent manner, which may explain its ability to
11 successfully persist within the hospital environment.

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1 Introduction

2 Fungal infections affect more than a billion people, resulting in approximately
3 11.5 million life-threatening infections and more than 1.5 million deaths
4 annually. There have been significant strides made in tackling these infections
5 over the past decade, but the global impact of these measures has yet to be
6 realized [1]. An important fungus worth consideration in this context is the
7 multidrug-resistant yeast *Candida auris*, which has been increasingly
8 described as a major global concern and cause of major nosocomial outbreaks
9 [2]. The impact on transmission and infection control is substantial, so
10 understanding their mechanisms of spread and survival in the hospital
11 environment is critical, particularly as it is able to persist on plastics and steel,
12 and survive as biofilms [3, 4]. Several recent investigations have established
13 that *C. auris* is capable of prolonged survival on surfaces [4, 5], and that
14 surface disinfection protocols had variable and unsatisfactory outcomes [5].
15 Given that it has been shown recently that 1000ppm of an active chlorine
16 solution is highly effective against these organisms tested in suspension [6],
17 then it is clear that surfaces play an important role in survival of this pathogen.
18 Our own work confirms this, with *C. auris* biofilms being generally insensitive to
19 a range of key antimicrobial agents, thus prolonging their survival capacity [3].
20 Therefore, identifying ways to minimise the impact of *C. auris* within the
21 hospital environment is imperative. The purpose of this study was to
22 investigate the general disinfectant sodium hypochlorite (NaOCl), commonly
23 used for terminal cleaning within the hospital environment, and the high level
24 disinfection agent peracetic acid (PA), on different substrate surfaces. These
25 data will support our understanding of how *C. auris* responds to different levels
26 of challenge on surfaces representative of the hospital environment.

28 Material and Methods

29 Strains and culture conditions

30 Throughout this study four *Candida auris* (Ca) isolates obtained from various
31 clinical sites [7], (NCPF 8971, NCPF 8973, NCPF 8977, NCPF 8978) were
32 used, as previously described [3]. All isolates were identified by ribosomal DNA
33 (rDNA) gene sequencing or matrix-assisted laser desorption ionization–time of
34 flight (MALDI-TOF) [7]. *Candida glabrata* (Cg) ATCC 2001 and *Candida*

1 *albicans* (Ca) ATCC 10231 was used as reference strains. All strains were
2 stored and maintained on Sabouraud dextrose (SAB) agar (Oxoid, Hampshire,
3 UK) prior to propagation in yeast peptone dextrose (YPD) (Sigma-Aldrich,
4 Dorset, UK) medium overnight at 30°C. Cells were prepared according to a
5 modified version of the British Standards for chemical disinfectants and
6 antiseptics [8]. Briefly, cells were washed by centrifugation in phosphate
7 buffered saline ([PBS] Sigma-Aldrich, Dorset, UK), and standardised to 1×10^7
8 cells/mL in sterile water containing 5% foetal bovine serum to simulate organic
9 material.

11 *Surface disinfection testing*

12 The following test surface substrates were used: cellulose matrix (IPS
13 Converters, Oldham, UK [1.25cm²]), 304 stainless steel (LaserMaster,
14 Redruth, UK [3.14cm²]) and Thermanox™ polyester coverslips (Fisher
15 Scientific, Loughborough, UK [1.32cm²]). Following the adhesion phase, non-
16 adherent cells were removed by washing with 1 mL PBS. Next, each surface
17 was challenged with either NaOCl (1000 and 10000 ppm, [Fisher Scientific,
18 Loughborough, UK]) or PA (2000ppm [Acros Organics, Geel, Belgium]), both
19 agents diluted to their respective working concentrations in sterile water.
20 Following 5 min or 10 min exposure, disinfectants were neutralised with 5%
21 sodium thiosulphate (Fisher Scientific, Loughborough, UK) for 15 min. The
22 neutraliser alone did not have a detrimental impact on *Candida* viability when
23 treated in the absence of a disinfectant (data not shown). Substrate sections
24 were then sonicated at 35 kHz for 10 min in sterile H₂O to remove cells, and
25 serial ten-fold dilutions in sterile water were plated on to SAB agar according to
26 the Miles and Misra plate count method. These were then incubated at 30°C
27 for 48 h. Parallel experiments were also performed to assess the potential for
28 regrowth following disinfection procedures. After treatment and neutralisation
29 as described above, test coupons were replaced in 10mL of fresh YPD media
30 and incubated for 24 h at 30°C with gentle of 100 rpm. Substrate adhered *C.*
31 *auris* cells treated with sterile water acted as a positive control, with substrates
32 containing no *C. auris* cells included as negative controls throughout this study.
33 After 24 h, the optical density readings were measured at a wavelength of

530nm (OD₅₃₀) using a microtitre plate reader (FluoStar Omega, BMG Labtech, Aylesbury, UK).

Statistical analysis

Data distribution, statistical analysis and graph production was performed using GraphPad Prism (version 7; La Jolla, CA, USA). Student t-tests were used to compare treated and untreated samples. A one-way analysis of variance and post-hoc Tukey test was used to compare the effectiveness of each disinfectant against the 3 different substrates. All experiments were performed in triplicate on three independent substrates, with the mean of each experiment used for analyses. Statistical significance was achieved if $p < 0.05$.

Results

Initially, a standard disinfectant challenge was performed against *C. auris* on different substrates relevant to the hospital environment. A cellulose substrate was included to act as control for porosity. It was shown that all four *C. auris* were significantly killed by NaOCl challenge at 1000 and 10000ppm, irrespective of substrate and strain, though differences were observed between these substrates. Complete eradication (100%) was only achieved on the cellulose substrate (Fig 1A). On the non-porous materials, significant quantities of viable yeast cells were killed on the steel surface following NaOCl at all treatment parameters, with an approximate 2.5 log₁₀ reduction ($p < 0.001$), with no significant differences observed at each time point and concentration tested (Fig 1B). Notably, those isolates treated with 1000ppm for 5 min showed significantly more regrowth compared to the other test conditions ($p < 0.001$). When *C. auris* was tested on a polymer substrate it was shown that 5 min exposure at 1000ppm was the least effective overall, and although there was significant activity observed (mean log₁₀ reduction = 1.29; $p < 0.001$), 4.95 log₁₀ was retained on the surface (Fig 1C). However, following an increased contact time of 10 min or increased concentration of 10000ppm, significantly enhanced activity was observed compared to the 5 min contact time ($p < 0.001$), with an approximate overall 3.5 log₁₀ reduction. When comparing both increased treatment parameters, no significant differences were observed between the regimens ($P = 0.347$), and no notable regrowth was detected.

Following a standard disinfection challenge, the efficacy of the HDL agent PA was assessed. When tested against 2000ppm of PA, it was shown that all *C. auris* isolates were significantly killed by this agent. However, differences were again detected between substrates. As observed with NaOCl, complete eradication (100%) was achieved on the cellulose matrix (Fig 2A), with this same fungicidal activity also observed on the polymer substrate (Fig 2B). However, compared to the other two substrates, significant quantities of viable cells were recovered from the steel substrate following PA challenge (mean $\log_{10} = 3.19$; $p < 0.001$), with an overall $2.70 \log_{10}$ reduction ($p < 0.001$) (Fig 2C). When re-inoculated into media post-challenge, substantial regrowth was recorded from both steel and polymer substrates, with minimal quantities recovered from the cellulose substrate.

For both disinfectants on each of the substrates, no differences were observed between strains, and both exhibited a similar profile to *C. glabrata* and *C. albicans*. Similarly, the presence of BSA was shown to have no effect of any treatments compared to no BSA controls. Liquid suspension tests showed that NaOCl and PA were highly effective at <20ppm and 40ppm, respectively.

Discussion

Although the precise mechanism of *C. auris* nosocomial transmission remains unknown, it is thought to be a multi-factorial process whereby it can colonise the environment and equipment of the healthcare setting. It has been reported to tolerate a number of environmental stressors, including temperature and salt, and some strains appear to have variable aggregative phenotypes that may have a role in persistence within the environment and the host [7, 9]. We therefore set out to investigate how resilient *C. auris* is within a controlled disinfection challenge using clinical isolates from the UK [7]. Here we report for the first time that both standard and high-level disinfection strategies were unable to completely eradicate *C. auris* from non-porous substrates.

Chlorine based disinfectants have variable yeasticidal activity against planktonic *C. auris* [6, 10], though their role in surface disinfection procedures

1 lacks definitive evidence. Recently, it has been shown that quaternary
2 ammonium compounds were poorly active against *C. auris*, whereas
3 environmental protection agency registered hospital disinfectants, such as
4 NaOCl containing solutions, were fungicidal on surfaces [5]. In a recent UK
5 outbreak, Schelenz and colleagues (2016) implemented chlorine based
6 disinfectants at 1000ppm three times daily for environmental cleaning, and
7 10000ppm for terminal cleaning [2]. The data presented herein support this
8 clinical guidance, though length of exposure at 1000ppm or an increased
9 concentration is an important factor for consideration to maximize *C. auris*
10 control. This is evident from our analysis of recovery of cultivable cells directly
11 following surface challenge at 1000 ppm for 5 min, which suggests this is an
12 ineffective control strategy.

13
14 It was interesting to note that we observed a significant difference in activity
15 when comparing the polymer to steel, which could be explained by *Candida*
16 species general ability to adhere and form biofilm, which are inherently more
17 resistant. This is reinforced by another study investigating these responses on
18 steel, where different exposure times and concentrations were reported to
19 effectively reduce *C. auris* viability by up to 6 log₁₀ [5]. Taken together, these
20 data suggest the standard disinfection procedures are surface dependent, and
21 given the diversity of fomites in the hospital setting then this could pose a
22 problem for disinfection. To this end we decided to explore a representative
23 high-level disinfection protocol. Here PA was used, a disinfectant routinely
24 used for endoscope reprocessing. Contrary to our previous data, it was shown
25 that on plastic polymers this disinfectant challenge was more effective,
26 showing significant reduction compared to stainless steel. This is the first
27 report to investigate this agent, and although it was used to represent a
28 superior disinfectant strategy, it revealed a risk for potential transmission via
29 contaminated endoscopes. *C. auris* has been isolated from a number of clinical
30 sources [9], so it is not unreasonable to suggest that this and other hospital
31 instruments could facilitate transmission.

32
33 Recent studies have suggested that *C. auris* has been shown to survive on
34 steel and plastic surfaces for 1 and 4 weeks, respectively [4, 11]. Comparison

1 of *C. auris* to *C. parapsilosis* persistence on plastics was quantified under
2 controlled hospital conditions (temperature and humidity). Low density test
3 suspensions (10^4) of *C. auris* was shown to remain viable (CFU counts) for up
4 to 14 days, though more sensitive esterase measurements suggested viable
5 activity up to 28 days that was comparable to *C. parapsilosis* [4]. Piedrahita
6 and colleagues (2017) further investigated *C. auris* in comparison to *C.*
7 *albicans*, *C. glabrata* and *C. parapsilosis*, specifically looking at moist and dry
8 inoculums over 7 days. Here they demonstrated that in moist conditions all
9 species were recovered in near maximum efficiency after 7 days, whereas only
10 40% of the dried inoculum were recovered on the steel substrates, which was
11 significantly greater than *C. albicans*, though *C. glabrata* and *C. parapsilosis*
12 were recovered by approximately 65% [11]. This is in line with our own
13 findings, showing comparable disinfectant sensitivity profiles for both *C. auris*
14 and *C. glabrata*. Nevertheless, given the multi-drug resistance phenotype of *C.*
15 *auris* compared to other species then its ability to persist is particularly
16 alarming.

17
18 While this study provides a useful insight into potential complications with
19 disinfectant procedures, there are some **limitations**. We have only been able to
20 test a limited panel of *C. auris* strains, although they did demonstrate similar
21 sensitivity profiles across each tested **parameter**. Furthermore, we only studied
22 two disinfectants at individual concentrations and contact times. Future studies
23 aim to undertake extensive analysis with commercial products in conjunction
24 with up-to-date infection control guidelines. Overall, this study reveals the
25 potential deficits we have in controlling this emerging fungal pathogen, and
26 only through understanding the biology of this multi-resistant pathogen will
27 assist us in devising new therapeutic and control interventions.

28 29 **Acknowledgements**

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Figure 1. Efficacy of sodium hypochlorite on *Candida auris*, *Candida glabrata* and *Candida albicans* on three different substrates.

Cellulose matrix (A), stainless steel (B) and polymer (C) were inoculated with 1×10^7 cells/mL of *C. auris* (NCPF), *C. glabrata* (CG) and *C. albicans* (CA) for 90 min before being treated with 1000ppm NaOCl for 5 min, 1000ppm for 10 min and 10000ppm for 5 min. Viable cells were then quantified by CFU (left hand y-axis) and regrowth was measured spectrophotometrically (right hand y-axis). Data represents means \pm standard deviation of triplicate datasets, with CFU \log_{10} reduction of each test substrate normalised to 1cm^2 . # indicates complete eradication compared to untreated control. N.A. = not applicable.

Figure 2. Efficacy of PA on *Candida auris*, *Candida glabrata* and *Candida albicans* on three different substrates.

Cellulose matrix (A), stainless steel (B) and polymer (C) were inoculated with 1×10^7 cells/mL of *C. auris* (NCPF), *C. glabrata* (CG) and *C. albicans* (CA) for 90 min before being treated with 2000ppm PA for 5 min. Cell viability (left hand y-axis) and re-growth (right hand y-axis) were quantified by CFU and spectrophotometric readings, respectively. Data represents means \pm standard deviation of triplicate datasets, with CFU \log_{10} reduction of each test substrate normalised to 1cm^2 . # indicates complete eradication compared to untreated control.



